

*Experimental Researches on Vegetable Assimilation and Respiration. VIII.\*—A New Method for Estimating the Gaseous Exchanges of Submerged Plants.*

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INTRODUCTION.

The method in universal use for evaluating assimilation in submerged plants consists in counting or measuring the evolution of bubbles of gas. This method gives satisfactory results in medium conditions, but fails at both extremes of the conditions—light, temperature, and  $\text{CO}_2$ -supply—which chiefly control the magnitude of assimilation. Critical work over a wide range of conditions is therefore impossible with it.

A really satisfactory method must take account of the alteration of the dissolved gases as well as of those that are liberated as gas-bubbles; because, when assimilation is slight, the oxygen formed may all dissolve in the water and no bubbles appear. When temperature is high the bubbles consist partly of other gases physically liberated from the water, and when the  $\text{CO}_2$ -content of the surrounding water is very high the bubbles escaping will consist chiefly of this gas.

Wishing to extend our researches from land-plants to water-plants, we have elaborated a method which is open to none of these objections, and which will be described in the present paper. In this method a continuous current of water containing dissolved  $\text{CO}_2$  flows over the assimilating plant, and the difference in the  $\text{CO}_2$ -content of the water before and after contact with the plant is a measure of the assimilation taking place. Fewer bubbles are liberated under these conditions than when the water is stationary, but the gas given off is collected automatically, analysed and allowed for in estimating the total assimilation.

The plant is contained in a glass chamber, and the conditions of illumination and temperature and  $\text{CO}_2$ -supply are completely under

\* The first six contributions of this series have appeared in the publications of the Royal Society; No. VII, by Miss A. Irving, on "The Beginning of Photosynthesis and the Development of Chlorophyll," will be found in the 'Annals of Botany,' October, 1910.

control, so that the interaction of these different factors can be directly investigated.

The results obtained by this method in a critical investigation of the relation between CO<sub>2</sub>-supply and assimilation will occupy the next paper of this series.

#### Section I.—THE APPARATUS.

The apparatus is schematically represented in fig. 1. The current of CO<sub>2</sub>-laden water flows by gravitation from the bottle A to the plant chamber B situated in the bath C; and, after passing up through it, thence by the route *d*, Y, *n*, *k*, into the lower end of one or other of the 200 c.c. pipettes D and E, finally escaping from them by the overflow tubes above into the measuring cylinders F or G.

The different parts of the apparatus call for description in some detail.

(a) *The Plant Chamber*.—The water-plant used is confined in a flat vertical glass-fronted chamber of oval outline, 18 cm. long and 11 cm. wide, the rim of which is formed by a wrought-iron frame 14 mm. square in section (for front view see inset to fig. 1). The iron rim is closed up to form a chamber by oval glass plates, back and front, which are luted firmly to it by a wax mixture of resin, beeswax, and vaseline. The back plate is waxed down permanently, but the front one is removed each time to put in material for a new experiment and then refastened.

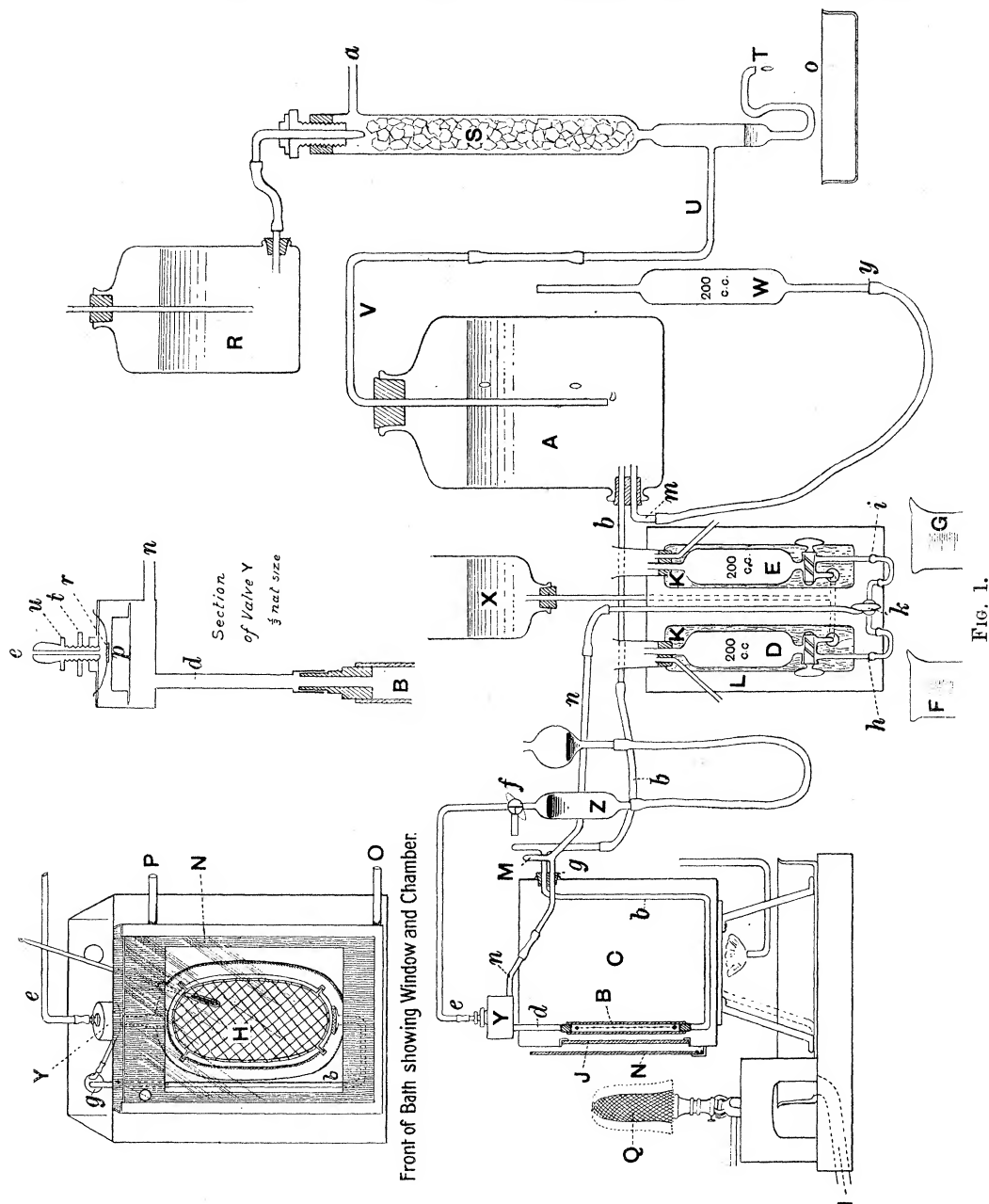
The chamber contains a removable oval grid H (made of silver wire, with meshes about 6 mm. broad) which lies against the back of the chamber, and to this the plant material is tied down with thread. The grid, the iron frames, and all metallic parts in contact with the water current, are coated with wax to prevent chemical action and possible poisoning of the plant.\* The iron frame of the chamber is pierced by an inlet tube (*b*) at its lowest point, and by an outlet tube (*d*) at its highest point. Close to the latter is a hole for a thermometer by which the internal temperature is determined. Immediately above the inlet tube is a thin sieve-like "baffle-plate," which stretches right across the opening and causes the inflowing water to spread out in all directions, hindering it from rising straight up the middle of the chamber.

(b) *The Water-Current*.—The effective fall which determines the rate of flow of the water-current through the apparatus is the difference in level between the mouth of the central tube of the Mariotte's bottle A and the mouth of the upper ends of the pipettes D and E where the water overflows.

It will become obvious that a uniform rate of flow is of fundamental

\* All parts are of silver or iron; no copper or brass is present.

importance in this method of investigation; the rate aimed at was about 300 c.c. per hour. The actual rate was continually determined by measuring



the amount overflowing from the pipettes into the graduated cylinders F and G. Any significant departure from uniformity was corrected by

raising or lowering the pipettes, by sliding up or down on the board L the slips of wood K to which the pipettes are attached.

Most of the disturbances in the rate of flow were due to the liberation of tiny bubbles from the liquid in the effluent tube (*n*). The impediment to flow which they caused was removed by shaking the tube and working the bubbles up into the blind arm M.\*

(c) *The Bath and Temperature Regulation.*—The temperature of the cubical copper bath of water in which the chamber was entirely immersed was kept constant by a thermostat when temperatures above that of the laboratory were wanted. (Only the burner below the bath is shown in the figure.) Temperatures between those of the laboratory and the water supply could be kept constant merely by a regulated circulation of water through the bath. The lowest temperatures used were obtained by careful regular additions of small quantities of ice.

In the front of the bath is a large glass window J for lighting the assimilation chamber, and in front of the window a screen of rapidly-circulating cold water to cut off the heat of the illuminating burner Q from the bath. This screen is made by a rectangular brass frame fastened to the front of the bath and carrying a second glass plate N. The space between J and N is full of water; O is the inflow and P the outflow for this water screen.†

(d) *Light for Assimilation and its Regulation.*—All the experiments were carried out with a special artificial light, that of the triple Keith high-pressure burner used in this laboratory since 1903. The burner Q was placed on a small wooden table fixed in front of the bath. The intensity of the light is determined by its distance from the assimilating plants. When the fronts of the nearest pair of mantles are 130 mm. from the front of the assimilation chamber, the arbitrary value of 6 has been assigned to the light-intensity.‡ From this standard the intensity in other positions can be calculated.

(e) *The Supply of CO<sub>2</sub> Solution.*—Tap-water that had been boiled for some time to remove the CO<sub>2</sub> and the calcium carbonate was used as being more

\* There is a small omission in fig. 1. The tube rising vertically from *k* should be continued up to a free end closed by a tap, used for sucking out obstructing bubbles, and the tube *n* should be attached to a lateral branch below the tap. Further the part of tube *b*, *b* represented as rubber should really be all glass.

† The screen carries about 1 inch thickness of water, and the front glass is not cemented in watertight, but pressed firmly against the metal rim all round by corks between it and the bath. It is therefore easy to replace if cracked, and the very moderate leakage trickles into the trough below the bath and is carried away at I.

‡ See Matthaei, 'Phil. Trans.,' B, 1904, p. 59.

wholesome for the plants than distilled water. A stock of this was saturated with  $\text{CO}_2$  which had been carefully scrubbed free from  $\text{HCl}$ , and diluted with more boiled tap-water to obtain the various strengths needed. Water cannot be saturated with  $\text{CO}_2$  by merely bubbling the gas through it, even in a very long time; prolonged violent shaking of the water with a large volume of pure  $\text{CO}_2$  will, however, achieve this.

The properly diluted  $\text{CO}_2$ -solution is filled into the bottle A whence it can flow to the chamber B by the tube *b*. As the solution flows out of the bottle air is drawn in down the central tube V, and on the principle of "Mariotte's bottle," the rate of outflow is constant and independent of the level of the liquid in the bottle.

If laboratory air were to be drawn in this way into the bottle it would carry some of the  $\text{CO}_2$  out of solution with it to the top of the bottle and the liquid would get progressively weaker. To prevent this the incoming air is charged with just as much  $\text{CO}_2$  as should be in equilibrium with the solution of  $\text{CO}_2$  in the bottle, and alteration of the strength is thereby prevented. This addition of  $\text{CO}_2$  to the air-current is accomplished by making the air traverse a  $\text{CO}_2$ -generating tower S, on its way to the bottle.

Hydrochloric acid of determined strength drops regularly from R on to the marble in this tower, trickles down and flows away below as a neutral liquid at T. The air entering above at (*a*) carries away the  $\text{CO}_2$  generated and is thus enriched to the desired amount before it leaves by the tube U. Minor adjustment of the amount of  $\text{CO}_2$  generated may be made during the course of an experiment by altering the rate of flow of the  $\text{HCl}$  by raising or lowering the dropper of the acid bottle by means of a screw attachment at the top of S.

The strength of acid required for any experiment was determined empirically and it was found that this whole procedure was quite satisfactory for maintaining constancy of  $\text{CO}_2$ -content in the water flowing into the assimilation chamber. The constancy attained in most experiments is shown in the successive analyses of samples withdrawn at *m* at regular intervals of time.

(f) *Estimation of the  $\text{CO}_2$  in the Solutions.*—The fundamental proceeding of the whole method is the determination of the amount of dissolved  $\text{CO}_2$  in (1) a sample of the liquid flowing from A to the assimilating plant in its chamber B and in (2) a sample of the effluent in D or E after it has passed over the plant. The samples analysed were always 200 c.c. of the liquid. For (1) a sample was drawn from the supply bottle by the tube *m* into a 200 c.c. pipette W at any given time and for (2) the 200 c.c. of liquid accumulated in pipette D or pipette E through which the effluent had been

flowing were taken. This liquid can be drawn out by switching the current into the other pipette by the three-way tap *k* and then disconnecting the rubber joint at *h* or *i*.

Above the analytical bench on a high shelf are set two 30-litre bottles containing N/10 standard baryta and N/20 HCl\* having burettes in connection with them, one to the baryta, and two to the acid for two simultaneous analyses. The procedure for estimating the dissolved CO<sub>2</sub> is to introduce 25 or 50 c.c. of the standard baryta into a clean glass cylinder of about 500 c.c. capacity and to take this to the apparatus and draw off into it the 200 c.c. of CO<sub>2</sub>-solution. The cylinder is brought back and corked up in air-tight connection with one of the burettes of N/20 HCl and the excess of free baryta titrated by this acid, using phenolphthalein as indicator and stirring by means of a current of CO<sub>2</sub>-free air forced through the liquid. The titre of the original 25 or 50 c.c. of baryta being known, the CO<sub>2</sub>-content of the 200 c.c. of the liquid is obtained in equivalents of N/20 HCl, and the weight of CO<sub>2</sub> in 100 c.c. of liquid is calculated.

(g) *Method of Securing Uniform Flow of Effluent.*—In maintaining the regular flow of the current a special difficulty arises from the fact that the outflow pipettes are at intervals completely emptied of their liquid for analysis. Should the flow be switched back to an *empty* pipette when it is desired to analyse the contents of the other pipette then, of course, there would be a sudden rush of the current owing to the whole height of the pipette being added to the effective fall which governs the rate of flow. It is therefore essential that, when the circulation liquid has been withdrawn for analysis, the pipette be filled up with some liquid or other before the current can be safely turned back into it without disturbing acceleration of rate.

For this purpose there is a reservoir of liquid X from which either pipette can be quickly filled by turning its controlling tap. This liquid is water containing about 7 per cent. of alcohol and tinged with methylene blue. Its lower specific gravity allows this accessory liquid to float up in the pipette on top of the inflowing stream of the proper solution without mixing with it. The colour enables one to see that there is a sharp line of separation kept between the two liquids and also to follow the rise of the accessory liquid and tell when the whole of it has been driven out at the top of the pipette, and the contents again consist only of the proper effluent from the chamber suitable for making another analysis.

If the contents of each pipette were withdrawn for analysis as soon as the blue liquid was all driven over then none of the actual liquid from the

\* In later years N/5 baryta and N/10 HCl have been employed.

chamber would ever pass away into F and G, but the whole of it could be analysed for its CO<sub>2</sub>-content.

As the current rate is 300 c.c. per hour and each pipette holds 200 c.c., this would mean an analysis about every 40 minutes. In practice an analysis about every hour is thought sufficient, and for the other 20 minutes the current solution drops over into the measuring cylinders.

(h) *Analysis of Gas-bubbles liberated by the Plant.*—When the CO<sub>2</sub>-content of the current through the assimilation chamber is low there may be an almost complete absence of “bubble-formation”; all the oxygen formed being carried off in solution in the liquid. With more active assimilation bubbles are liberated in the chamber; these are never pure oxygen, but contain always CO<sub>2</sub> in proportion to the CO<sub>2</sub>-pressure in the liquid.

It follows that just as in strong CO<sub>2</sub>, bubble-counting alone gives an exaggerated value of assimilation; so also analysis of the diminution of CO<sub>2</sub> in the liquid alone gives too great a value for assimilation, because some of the CO<sub>2</sub> gone from the solution has really diffused into the gas-bubbles that are forming. For the correct measure of assimilation this physical loss must be estimated and subtracted from the total apparent loss of CO<sub>2</sub>.

To provide for this the gas-bubbles given off from the plant are separated from the liquid, continuously collected and analysed at appropriate times by means of the valve Y and the gas collector Z. The water-current leaving the top of the assimilation chamber by *d* passes immediately through a hollow metal drum Y on its way to the outflow tube *n*. This drum (see the large scale drawing of Y at the top of fig. 1) contains an outlet tube (*e*) in its roof, through which the gas that rises above the water, and the gas alone, is drawn off to the gas collecting vessel Z. Z is connected with a reservoir of mercury, which is lowered so that there is a strong suction exercised through *f, e* to the valved outlet in the top of the drum.

In the drum is the very light, loosely fitting, hollow metal float *p*. If the drum is full of water this float rises as high as possible and presses the little metal disc *r* (mobilely suspended by a strip of oiled silk) against the nozzle of the gas-outlet tube. This prevents any escape of water in the direction *e, f*, in spite of the suction from the mercury reservoir. When a certain amount of gas from the chamber has collected round the float in the top of the drum, the float sinks by its own weight, and the disc *r* drops and allows the gas to be sucked out through *e* till the rising water carries *p* and *r* up again to close the nozzle. The nozzle-tube *e* can be screwed down through the roof by the flange *u* till the valve mechanism is working well, and then locked in position by the screw collar *t*.\*

\* The roof of the drum is not soldered in but fits tightly into a rabbet (not shown),

This form of valve has worked quite smoothly and the gas given off passes, with an occasional drop of water, intermittently into the receiver Z. From here by turning tap *f* and raising the mercury reservoir the gas can be driven through the side tube into a eudiometer for analysis.

From the shrinkage in volume of the gas after treatment with KHO in the eudiometer the absolute amount of gaseous CO<sub>2</sub> physically liberated from the water-current during the time since the last analysis is determined: this is used as a correction to obtain the proper assimilation.

#### Section II.—THE APPLICATION OF THE METHOD.

(a) *Procedure in a Typical Experiment.*—As an example of the method pursued in carrying out an experiment, and the calculation involved in arriving at the real assimilation, we propose now to describe a single case in detail. We select the first experiment done in 1907 when the apparatus had taken on the final form just described. The object of this experiment was to determine the assimilation by *Elodea* with an illumination equal to 5·7, at a temperature about 20° C., and a CO<sub>2</sub>-supply of about 0·03 grm. per cent. (about one-sixth saturated).

The *Elodea* was gathered at 9.45 A.M. on June 7th in a stream about a mile from the laboratory, brought back in a tin and placed at once in tap-water. The first proceeding is to set up the material in the assimilation chamber.\* Only the greenest and healthiest shoots were selected from the gathering, and these were laid down one by one on the silver grid until a continuous dense green layer, without any gaps, was formed on the front side of the grid. The whole was secured with cotton, the grid slipped into the chamber (its four projecting pegs fitting into slots in the iron frame), and the front glass, which had been carefully cleaned since the previous experiment, was again waxed on.

To accomplish this the glass was pressed down on to the film of wax left on the metal frame, and then lumps of wax-mixture were placed round the edge at intervals. These were melted and run uniformly round with a tiny gas-flame from a capillary tube at the end of a length of flexible tubing, the junction being waxed over watertight. The drum can, therefore, be easily opened for inspection and adjustment. The strip of oil silk emerges through fine slits in the roof, which are also closed by wax.

\* At the end of each experiment the chamber is opened by taking off the front glass, wiped and left to dry; the silver grid is removed, and the plant carefully taken off and spread out to dry up quickly in the air in order to obtain its dry weight. The bent glass inlet tube below, *b*, and the valve-drum Y above are never detached from the chamber. The widened lower end of the tube of the drum just slides over the metal outlet tube of the chamber, and so makes a rigid joint which indiarubber tube renders watertight (see large scale drawing at top of fig. 1).



chamber being kept horizontal. It may take some minutes to make a completely air-tight joint all round, and the application of the gas-flame may warm the chamber up a few degrees, but as there is a thermometer inside, it can be seen that the temperature reached is not injurious. During this time there is no water inside and, before admitting water, the chamber is tested for leaks by a gauge.

The chamber is then adjusted upon a tall brass stand (not shown in the figures, but similar to the one figured in "Assim. and Resp.," III, p. 57), and the whole lowered into the water-bath, previously regulated to the desired temperature. The position of the stand is shifted till the front of the chamber is 4 cm. behind the glass window of the bath, and the appropriate connections made at (*e*), (*n*), and (*g*).

The chamber is now to be filled up\* with the proper CO<sub>2</sub>-containing liquid which has been previously made and transferred into the supply bottle A. This is done by disconnecting the outlet tube *e* just above the valve Y and sucking till the solution first fills the chamber and then the valve and tubes. As soon as the tap *k* is turned to one of the pipettes D or E, the flow will commence. In this particular experiment this was done at 11.19 A.M., and pipette D (previously filled up with blue liquid) then began to overflow above at a steady rate, the overflow liquid dropping into the litre cylinder F. Illumination of the plant was begun at 11.25 by lighting the triple high-pressure burner (placed 13.4 cm. from the front of the chamber), and the circulation of water through the water-screen in front of the bath was started at the same time.

Everything has now been arranged, and the experiment starts its course at 11.25, see Table I on p. 383. It is obvious that, with a slow flow through the chamber, it will be some time before the contents of the pipette D represent the full effect of assimilation in removing CO<sub>2</sub> from the liquid. With rates about 300 c.c. per hour, the current must flow for more than an hour before it is worth while analysing the effluent.

In this experiment, as the table shows, the first sample of the effluent was withdrawn at 12.45, the current being then switched through pipette E already full of blue liquid. The CO<sub>2</sub> left in the 200 c.c. of liquid from D was still equal to 22.18 c.c. N/10 HCl, and comparison with the later numbers in the column shows that the full effect had not then been reached. The experiment was continued till 7 P.M., and six more analyses of the effluent were made, about one every hour, each representing the liquid flowing in 40 minutes. About once an hour also an analysis was made of the *affluent*

\* Before doing this, the dropping of acid on to the marble in the tower is started and adjusted to the appropriate rate known from previous experience.

Table I.—Experiment 1, June 7, 1907. *Elodea canadensis*, Dry Weight = 1.955 gm. Temp. 20°·3 C. Light at 13.4 cm., i.e. (L.I. = 5.7).

CO <sub>2</sub> -content of 200 c.c. of current solution, expressed in c.c. N/10 HCl-equivalent.			Current rate, by outflow into measuring cylinders.			Temperature.			CO <sub>2</sub> carried off in gas-bubbles.
Time.	Affluent.	Effluent.	Time.	F.	G.	Time.	Bath.	Chamber.	
11.19 A.M. current started— 11.44—11.46 12.5—12.45 12.43—12.45 1.2—1.42 1.40—1.42 1.46—3.6 3.4—3.6 3.49—4.9 4.7—4.9 4.46—5.6 5.4—5.6 5.46—6.6 6.4—6.6 6.40—7.0 6.58—7.0	— [25.09] 26.31 26.14 25.59 25.36 25.98 25.58 25.46	— [22.18] D 19.45 E 19.04 D 19.14 E 19.22 D 18.58 E 18.55 D	11.19 12.45 1.42 3.6 4.9 5.6 6.6 7.0	464 [324] 461 (329) 306 (322) 292 (324)	303 (319)  310 (295)  306 (306)	11.45 12.4 12.21 1.5 1.40 1.55 3.6 3.58 4.28 5.27 6.15 7.2	19.8 20.0 19.6 19.0 20.0 19.8 19.8 19.6 19.5 19.8 19.9 20.0	20.3 20.5 20.4 19.8 19.7 20.5 20.4 20.3 20.2 20.4 20.5 20.6	11.25 A.M. to 3.36 P.M., 4.8 c.c. CO <sub>2</sub> = 1.2 c.c. per hour. — 3.36 P.M. to 6.0 P.M., 3.6 c.c. CO <sub>2</sub> = 1.5 c.c. per hour.
Averages.....	25.79	19.00	Per hour 316.5 c.c.			Average.....			1.3 c.c.

solution by collecting 200 c.c. slowly (in two minutes) in the pipette W, and estimating its CO<sub>2</sub>-content as described in Section I, f.

As in theory every particle of liquid takes about one hour to pass right through the apparatus, so any sample of effluent should strictly be compared with the sample of affluent taken an hour previously. When the composition of the affluent keeps fairly constant, as in this experiment, this point is not important, but if the affluent is altered in CO<sub>2</sub>-content, either intentionally or by failure in the CO<sub>2</sub>-generating tower, then it is important to bear this relation in mind.

For this particular experiment, only the *average* assimilation during six hours of the selected conditions is required, and the averages of the affluent and of the effluent liquids are taken, neglecting the first reading in either column of the table.

(b) *Data Required for an Exact Evaluation of the Assimilation.*—The magnitude of the following eight factors must be known numerically before a precise statement can be put forward about the assimilatory activity of the plant: (1) the weight of plant; (2) the intensity of illumination; (3) the temperature in the chamber; (4) the percentage of CO<sub>2</sub> in the circulating fluid; (5) the difference between the CO<sub>2</sub> in the affluent and effluent; (6) the rate of the current; (7) the CO<sub>2</sub> lost as gas-bubbles in the chamber; and (8) the magnitude of the plant's respiration.

We must say yet a little about the determination of each of these magnitudes in this particular experiment of June 7, 1907, before we pass to the calculation of the final result.

The unit of comparison for all cases and all plants in this method is the *quantity of leaf that will cover an arbitrary surface*, here 137 sq. cm., which is the area of the silver grid. In such a plant as *Elodea* the ideal unit, a unit area of the leaf-tissue itself, cannot be employed: one can only pack the shoots so closely (there is no objection to overlaying) that there are no gaps left where light is not being arrested, and then, however much leaf there is shaded and wasted as regards assimilation, there is yet the unit area of chlorophyllous cells exposed to the full illumination on the surface of the matted mass.

(1) The weight of plant required to cover the grid varies with its habit. It must in each case be ascertained in order to make the necessary allowance for the respiration, which varies with the total weight employed. As water-plants cannot be surface-dried satisfactorily, we have not taken the fresh weight, but have air-dried the material after each experiment, and have based the allowance for respiration on this dry weight.

(2) The standard light, at 13.4 cm., was used, having the arbitrary intensity 5.7 (see Section I, d).

(3) The temperatures of the bath and of the inside of the chamber were noted at intervals, as the entries in Table I show. The latter is always a little higher than the bath, due to the non-assimilatory radiation absorbed by the green leaf. The average temperature of the chamber, throughout the experiment, was 20°·3 C.

(4) The richness in CO<sub>2</sub> of the medium which bathes the leaves is of great importance. The number adopted must be the mean between the CO<sub>2</sub>-content of the liquid entering the chamber and of that leaving it. The average of the last seven readings for the affluent gives 25·79 c.c. N/10 HCl as the content of 200 c.c. of the liquid entering the chamber. The corresponding number for the effluent is 19 c.c. So the mean content of the chamber equals 11·20 c.c. N/10 HCl per 100 c.c. of liquid = 0·0279 grm. CO<sub>2</sub> per cent. (1 c.c. standard acid being equivalent to 0·00249 grm. CO<sub>2</sub>).

(5) The difference between the CO<sub>2</sub>-content of the affluent and effluent gives in its uncorrected form the measure of the CO<sub>2</sub> which has disappeared from the chamber, chiefly, of course, by photo synthesis in the green tissues. Here it is 6·79 N/10 HCl = 0·01693 grm. CO<sub>2</sub> per 200 c.c. of solution, and this figure has to be calculated per hour (6), and corrected for bubbles (7) and respiration (8).

(6) The current rate is measured throughout the experiment by collecting all the effluent liquid in the measuring cylinders, F and G.

The outflowing liquid shifts from cylinder F to G every time the current is diverted from pipette D to E, and *vice versa*, so that the volumes of effluent recorded in the table come alternately in F and G at rather irregular intervals of time. The actual volumes in the cylinders\* are given in Table I and in brackets beneath each the rate per hour calculated from it. The average rate between 12·45 and 7 P.M. works out at 316·5 c.c. per hour. Therefore the weight of CO<sub>2</sub> lost per hour in the chamber is:—

$$0\cdot01693 \times \frac{316\cdot5}{200} = 0\cdot02679 \text{ grm.}$$

(7) The amount of CO<sub>2</sub> escaping into the gas-bubbles formed must be ascertained and subtracted from the value in (6).

The gas evolved as bubbles was automatically collected over mercury in the receiver Z from 11·25 A.M. throughout the experiment as shown in the last column of the table. At 3·36 the amount collected was passed into the eudiometer for analysis. At laboratory temperature and pressure its total volume was 37·3 c.c., of which 4·8 c.c. proved to be CO<sub>2</sub>; = 1·2 c.c. CO<sub>2</sub> per hour. At 6 P.M. the gas collected since 3·36 was again withdrawn and

\* The contents of the cylinders are read off at frequent intervals to see if sufficient constancy is being maintained; if not, readjustment is made.

analysed. Its total volume was 27.4 c.c., containing 3.6 c.c.  $\text{CO}_2 = 1.5$  c.c.  $\text{CO}_2$  per hour. Therefore the average correction to be applied for the whole experiment was taken at 1.3 c.c.  $\text{CO}_2$  per hour  $= 0.00239$  gram.  $\text{CO}_2$  (taking the weight of 1 c.c.  $\text{CO}_2$  at laboratory temperature and pressure as 0.00184 gram.).

(8) During the experiment  $\text{CO}_2$  is being formed in respiration both in the illuminated parts and in the shaded leaves in the background, *i.e.* by the whole *weight* of tissue present. Several separate experiments to determine the respiration were made at different temperatures, using the same procedure as in this experiment except that the bath was darkened. Some of these were made on *Elodea* before feeding up by assimilation, and others after several hours' preliminary assimilation, whereby the respiration is increased. The mean of the two values was taken as basis for a correction. The measurements of respiration were made at four different temperatures, and from these data a curve was constructed by which the respiration at any temperature within the range of the research could be derived.

After experiment the respiration plants were all dried and weighed air-dry and the respiration expressed per unit of dry weight. One gramme of dry *Elodea* has when fresh a respiration of 0.00125 gram.  $\text{CO}_2$  per hour at  $20^\circ\text{C}$ . Therefore the dry 1.955 gram. of *Elodea* used in this experiment involves a correction of 0.00244 gram.  $\text{CO}_2$  per hour.

(c) *Final Calculation of Assimilation.*—We have now the data for calculating the corrected *Real Assimilation* proper to 137 sq. cm. surface of illuminated *Elodea* under the conditions of the experiment, *viz.*, illumination  $= 5.7$ , average temperature of  $20^\circ\text{C}$ . and  $\text{CO}_2$ -pressure in solution of 0.0279 gram.  $\text{CO}_2$  per 100 c.c. (about 15 volumes of  $\text{CO}_2$  per cent.).

$\text{CO}_2$ disappearing in chamber per hour .....	0.0268
Subtract $\text{CO}_2$ passing away in gas-bubbles .....	0.0024
Apparent assimilation .....	0.0244
Add $\text{CO}_2$ calculated for respiration at $20^\circ\text{C}$ . ...	0.0024
Real assimilation .....	0.0268

The corrections for bubbles and respiration are not large under these medium conditions, and by chance they happen to be equal and antagonistic, so that the total correction has no effect. Of course, this will not be so with solutions richer or poorer in  $\text{CO}_2$ .

This final number for the *Real Assimilation* of *Elodea* under this particular combination of environmental factors must be regarded as being

very soundly established, as it is derived from the mean of six concordant hours of assimilation.

Each experiment of this kind is a whole day's work, but a considerable number have been put together in the years 1905, 1907, and 1908. The series dealing with the relation between assimilation and the  $\text{CO}_2$ -content of the liquid is fairly complete, and the results will be published in the next paper of this series.

(d) *Blank Experiments.*—We may conclude this section with a reference to one of the blank experiments made to test the accuracy and uniformity of the estimations. The course of this experiment is represented graphically in fig. 2.

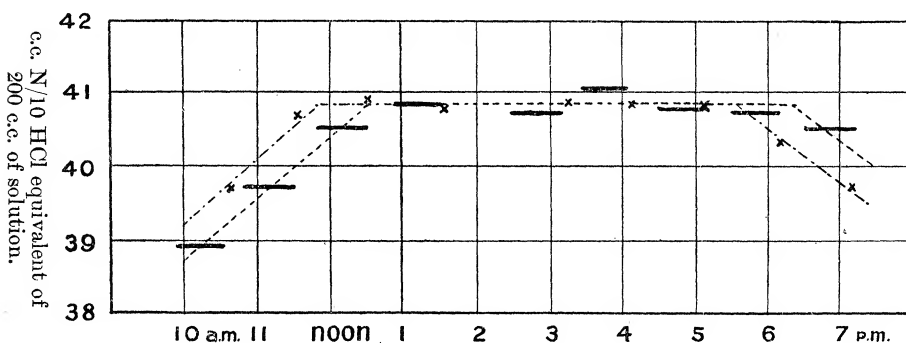


Fig 2

The procedure was exactly like that just described, but the chamber was set up empty, with no plant in it. The  $\text{CO}_2$ -solution prepared for the experiment was about quarter saturated, of such a strength that 200 c.c. of it equalled about 40.6 c.c. of  $\text{N}/10$   $\text{HCl}$ . The current was started at 9 o'clock (see fig. 2), and an estimation of the effluent was made every hour till 7 p.m. At the moment of the end of each collection of the effluent a sample of the affluent also was taken and analysed. Both sets of results are plotted in the diagram. Here the ordinates directly represent the equivalent in cubic centimetres of  $\text{N}/10$  acid. The series of heavy horizontal lines represents the effluent collected in the pipettes D or E; as each contains 200 c.c., or the flow during 40 minutes, the lines drawn are of a corresponding time-length. The small crosses are the analyses of the affluent placed at the times when they were withdrawn in the pipette W. In this experiment the  $\text{CO}_2$  solution was presumably not well mixed or the  $\text{CO}_2$  generator at fault, for the strength of the solution rose steadily from 9 A.M. till noon. From noon to 5 P.M. uniformity was maintained, after

which the strength of the solution fell off again. During the middle period the working is all that could be desired. The effluent and affluent analyses are practically identical all the time, which shows that there is no loss of  $\text{CO}_2$  during the long journey of the water current, and that the general procedure is satisfactory.

Irregularities of current rate do not come into play in this form of experiment, and we reckon that the errors of a real experiment are bigger than this blank indicates, and may amount to perhaps 1 per cent., which is satisfactory enough.

The divergence of the effluent and affluent values at the two ends of the diagram as here represented is illusory, because it is merely due to the lag in the effluent on account of the hour or more it takes to flow through the chamber and apparatus. If the values of the affluent were all moved on an hour in the diagram they would coincide with those for the effluent right through the whole experiment. This blank experiment has been chosen because it illustrates the lag so well, which varies in different experiments from 40 to 80 minutes.

It must be borne in mind that it is not proposed to obtain critical values when the concentration of the  $\text{CO}_2$  is rising or falling. Only when the series of analytical values of the affluent is nearly constant is the experiment considered a trustworthy one.

In conclusion we should mention that the cost of the important parts of the apparatus was provided by a Government grant from the Royal Society.

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